ABSTRACT OF THE DISCLOSURE

[41] The present invention provides a simple and rapid method for site-directed mutagenesis of more than, for example, 10 sites simultaneously with up to 100% efficiency. The method uses two terminal tailed primers, specific for each end of the gene (or DNA sequence) to be mutated, with a unique nucleotide tail each that are simultaneously annealed to template DNA together with a set of mutagenic primers in-between. Following synthesis of the mutant strand by primer extension and ligation with, for example, T4 DNA polymerase and ligase, the unique mutant strand-specific tails of the terminal primers are used as anchors to specifically amplify the mutant strand by high-fidelity polymerase chain reaction. Furthermore, specific restriction endonuclease sites in the two anchor primer tails may be used for convenient subcloning of the PCR product in any desired cloning or expression vector (for subsequent sequencing or expression and functional studies of the mutated gene).